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(54) Title: DUAL DELIVERY OF METHOPRENE

(57) Abstract

Described is a method of preventing the propagation of fleas on a host dog or cat which comprises, providing adult fleas with nutrient blood which contains an ovicidally effective amount of methoprene, and exposing the adult fleas to ovicidally effective amounts of dermally deposited methoprene wherein said dermal deposit results from the secretion of methoprene from methoprene absorbed in the host's digestive tract.

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DUAL DELIVERY OF METHOPRENE

BACKGROUND OF THE INVENTION

Ectoparasitic infestation on domestic animals, especially fleas on dogs and cats, continues to be a problem, and while many methods are known in the art of controlling ectoparasites on domestic animals none of these are entirely satisfactory and they frequently suffer from one or more drawbacks. The present invention provides systemic control of ectoparasites by providing dual delivery of an insect growth regulator which involves both blood delivery and dermal delivery from subcutaneous fat deposits from the oral administration of the insect growth regulator..

Systemic control of animal parasites is accomplished by absorbing an active compound in the blood stream or other tissue of the host animal. Parasites which eat or come in contact with the active compound in the blood or tissue are killed either by ingestion or contact.

Accordingly an object of this invention is to provide a method for the use of certain insect growth regulators, and particularly methoprene to systmetically control domestic animal ectoparasites.

Another aspect of the invention is to provide a dual mechanism for preventing flea propagation on a host dog or cat by providing adult fleas with insect growth regulators that are transmitted by both the hosts blood and by dermal contact with skin secretions from the host which include the insect growth regulator.

This and other objects of the invention will readily become apparent to those skilled in the art.

SUMMARY OF THE INVENTION

Surprisingly, it has been found that juvenile hormone compounds such as methoprene administered orally or otherwise to a companion animal such as a dog or cat may be delivered to an ectoparasite in such a way as to expose the ectoparasite to both indigested methoprene and dermal methoprene. The juvenile hormone, methoprene is taken up from the digestive tract and transported by the blood in the subcutaneous fat layer. Within 24 hours after oral dosing analytically detectable quantities of methoprene are found on the fur of the host animal. Since

ectoparasites, particularly fleas living on the skin of the host animal also deposit their eggs on the skin of the host, the adult parental ectoparasite receives not only an oral dose of methoprene from the blood and intercellular fluids it ingests during its feeding activities, but also a dose of methoprene from exposure to the dermal quantities of methoprene secreted with the host animals skin oils.

Additionally newly laid eggs are susceptible to the effects of methoprene and receive a further dermal dose by coming into contact with the methoprene in the skin oils secreted by the sebaceous glands associated with the hair follicle.

The term ectoparasite as used herein has its normal meaning in the art and includes fleas, ticks, lice, mosquitoes, and other biting insects.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENT

In the method of the present invention, ectoparasites, particularly fleas are prevented from propagating on a host dog or cat by providing adult fleas with nutrient blood which contains an ovicidally effective amount of methoprene, and exposing the adult fleas to ovicidally effective amounts of dermally deposited methoprene wherein said dermal deposit results from the secretion of methoprene from methoprene absorbed in the hosts digestive tract.

Methoprene has larvicidal effects wherein the compound acts by disrupting fleas development to such an extent as to be fatal on larvae. And in common with other insect growth regulators it also has an ovicidal effect and prevents hatch and/or development of eggs when applied directly to adults. As used herein "ovicidally effective" means an effect which leads to a reduced rate of hatching of eggs or to the inability of the male to fertilize eggs resulting in sterile egg production

Exemplary insect growth regulators (IGR) such as chitin synthesis inhibitors, ecdysone agonists and especially juvenile hormones and juvenile hormone analogues and/or mimics which have been found to be most effective for treating ectoparasites, especially fleas, on dogs and cats are included in the invention. Examples include methoprene, hydroprene, kinoprene, fenoxycarb, fluphenacur, cyromazine, chlorfluazuron, triflumuron, pyriproxfen, and N-[[[3,5-dichloro-4-(3,4,5-trichloro-pyrazol-1-yl)phenyl]amino] carbonyl]-2-chlorobenzamide. Methoprene is well known in the art and has the formula (isopropyl (E,E)-11-methoxy-3,7,11-trimethyl-2,4-dodecadioenoate.

Some of the insect growth regulators mentioned above bear an asymmetric carbon atom and accordingly there are (R) and (S) enantiomers of these compounds.

As used herein (R,S) refers to the racemic mixture and (S) refers to the compound comprising a predominance of the (S)-(+) enantiomer. Where the compound name is used herein without reference to its enantiomeric content, the term is inclusive of both (R,S) and (S). The preferred active compound of the invention is methoprene and particularly (S)-methoprene.

Forms of application include oral, parenteral, implant, or as a bolus. Formulated means in the form of a powder, a tablet, a wafer, a granulet, a capsule an emulsion a gel, a foam or other composition suitable for administering an effective amount of active ingredient. The preparation may also be administered to the host animal indirectly for example it may conveniently be mixed with the animals feed.

A preferred mode of administration of the active compound includes oral application. Methods of oral application include but are not limited to compounds premixed in dog and cat food, fed in biscuit or treats, chewable tablets, water dissolvable capsules or tablets and the like.

The total dose for an active insect growth regulator ingredient may vary from one genus of animal to the other, and may even vary within the same genus because dosage depends on body weight among other factors. An adequate dose is in the general range from 0.01 to 800 mg/kg body weight of the host animal.

The present invention is also directed to a dual method of preventing the infestation of fleas on a host cat or dog which comprises administering to said cat or dog orally, parenterally or by implant an ovicidally or larvicidally effective amount of methoprene wherein said methoprene is transmitted to the fleas by the host's blood and by dermal contact with skin secretions of the host comprising methoprene.

The following example illustrates the invention without in any way restricting the same.

EXAMPLES

Sampling, Pretreatment Fleas and Eggs and Treatment - Fifteen dogs were pretreated with 100 adult cat fleas each and placed in individual flea egg collection caging and held for 24 hours to allow time for eggs to fall from the fur. These were collected and counted. Based on pre-treatment adult fleas counts, flea egg counts and health, twelve doges were selected for inclusion in the test. These dogs ranging in weight from 6.6 kg to 14.6kg and of both sexes were divided into four groups of three dogs per group. Group 1: the untreated control; Group 2: positive control,

received technical (S)-Methoprene (96.23%); Group 3: received 60% (S)-Methoprene - and Group 4: received 40% (S)-Methoprene. All treatments were administered as oral boluses at the rate of 50 mg/kg body weight equivalent technical (S)-methoprene, and groups 2, 3 and 4 received oral treatment with methoprene one time each at start (Time 0).

Sampling, Flea Egg Hatch -On days 3, 10, 17, 24, 31, 45, 59, and 80 all dogs in each group were infested with adult cat fleas *(Ctenocephalides felis)*. On days 6, 13, 20, 27, 34, 48, 62, and 83, all dogs in each group were placed in individual flea egg collection caging and held for 24 hours to collect flea eggs. The eggs from each dog were collected, sieved to remove hair, feces etc., and counted into 4 containers of 25 eggs per container. The eggs were held in rearing incubators for 72 hours then checked microscopically for hatch. Eggs from untreated dogs were collected first and held in a separate incubator from those eggs collected from treated dogs. All treatments produced greater than 99% control of flea egg hatch on Day 6 (Table 1) . On Day 13 the technical, 40% and 60% treatments provided 98.4, 94.7 and 91.4% control respectively. By Day 27, the percent control of egg hatch in the technical and 40% treatments was down to 72.5 and 74 percent control, respectively. The 60% treatment fell to 33.9 percent control on Day 27 and continued to fall to 0.0% control on Day 83. By Day 83 , the technical treatment was down to 3.4% control and the 40% treatment provided no control of flea egg hatch.

Egg hatch rate and percent control of hatch as illustrated in Table 1 were calculated for each group as follows:

$$\frac{\text{Total hatched}}{\text{Total eggs}} = \text{Hatch Rate}$$

Percent control (or inhibition) of hatch rate was calculated as follows:

$$\frac{\text{Hatch rate in untreated - hatch rate in treated}}{\text{Hatch rate in untreated}} \times 100$$

Larval mortality was documented 72 hours after egg collection on Day 62 and Day 83 (Table 2). Larval survival rate was calculated for each group as follows:

$$\frac{\text{Total live larvae}}{\text{Total eggs}} = \text{Larval Survival Rate (Survival)}$$

Percent control (or inhibition) of larval survival rate was then calculated as follows:

$$\frac{\text{Survival in untreated - survival in treated}}{\text{Survival in untreated}} \times 100$$

Considering that no larvae died in the untreated group (group 1), all larvae mortality in the treated groups was apparently caused by the treatments. Therefore, on Day 62, percent control of first instar larvae at 72 hours post-egg collection was 100 percent.

TABLE 1: Flea Egg Hatch and % Control of Egg Hatch

DAY	6	13	20	27	34	48	62	83
Group 1 Hatch Rate	0.88	0.95	0.92	0.90	0.89	0.77	0.81	0.61
Group 2 Hatch Rate	0	0.02	0.09	0.25	0.36	0.51	0.08	0.59
% Control	100	98.4	90.6	72.5	60.0	33.9	90.6	3.4
Group 3 Hatch Rate	0	0.08	0.54	0.60	0.61	0.74	0.72	0.83
% Control	99.5	91.4	40.9	33.9	30.8	4.0	10.3	0
Group 4 Hatch Rate	0	0.03	0.29	0.24	0.17	0.51	0.78	0.66
% Control	100	96.7	68.7	74.0	81.2	33.6	3.3	0

TABLE 2 - Larval Survival 72 Hours Post-Collection

Group #	Total Eggs	Hatch	Live Larvae	Survival Rate	Percent Control
DAY 62					
1	295	238	238	0.807	N/A
2	304	23	0	0	100
3	294	213	107	0.364	54.9
4	296	231	190	0.642	20.4
DAY 83					
1	302	184	184	0.609	N/A
2	289	170	139	0.481	21.0
3	294	243	224	0.762	25.1
4	299	196	171	0.572	6.1

Blood Sampling -. Prior to bolusing (time 0 hour), one dog from group one and all dogs in groups 2, 3 and 4 were bled. Using this same collection procedure, the same dogs were bled again at 0.5, 2, 12, 24, 48 and 72 hours post-treatment. Each blood sample consisted of 5 mls, and was placed in a heparinized vacutainer and immediately frozen after collection. Samples were analyzed for ppm of S-methoprene by a Enzyme Linked Immunosorbet Assay (ELISA) procedure. The average of the results for all the dogs in each group are shown in Table 3

TABLE 3 - Average of S-Methoprene in Blood (Concentration in ppm)

Time (hr)	Group 1	Group 2	Group 3	Group 4
0	0.00	0.00	0.00	0.00
0.5	0.00	13.05	12.16	41.94
2	0.00	10.3	8.02	65.93
12	0.00	1.56	1.68	8.29
24	0.00	0.25	0.44	2.71
48	0.00	0.10	0.04	0.30
72	0.00	0.01	0.01	0.10

Sampling Hair - Hair samples (5 gm) were clipped from all dogs on Days 9 and 66 and placed in labeled half pint freezer jars with aluminum foil seal under the lid. These samples were immediately frozen (-20C) and held in a freezer until further analysis. Results are shown in Table 4. The control, untreated did not reveal the presence of (S)-methoprene.

TABLE 4- Concentration of (S) -Methoprene ng/g in Hair Samples

		ng/g (S) -Methoprene	
Group No.	Dog	(Day 9)	(Day 66)
2	1	257	16
	2	--	41
	3	--	27
3	1	222	< PQL
	2	--	57
	3	--	11
4	1	316	8
	2	--	6
	3	--	68

PQL = Practical Quantitation Limit; PQL is 5 ng/g for group 4 and is 10 ng/g for group 3.

What is claimed is:

1. A method of preventing the propagation of fleas on a host dog or cat which comprises,

(a) providing adult fleas with nutrient blood which contains an ovicidally effective amount of methoprene, and

(b) exposing the adult fleas to ovicidally effective amounts of dermally deposited methoprene wherein said dermal deposit results from the secretion of methoprene from methoprene absorbed in the hosts digestive tract.

2. A method according to claim 1, wherein the methoprene is administrated orally.

3. A method according to claim 1, wherein the methoprene is S-methoprene.

4. A method according to claim 1 wherein the methoprene is given in a formulated dose.

5. A method of preventing the infestation of fleas on a host cat or dog which comprises administering to said cat or dog orally, parenterally or by implant an ovicidally or larvicidally effective amount of methoprene wherein said methoprene is transmitted to the fleas by the host's blood and by dermal contact with skin secretions of the host which includes methoprene.

6. A method according to claim 5 wherein the methoprene is (S)-methoprene.

INTERNATIONAL SEARCH REPORT

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A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 A01N49/00

According to International Patent Classification(IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 A01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EP 0 255 803 A (CIBA GEIGY AG) 10 February 1988 see the whole document ---	1-6
A	OLSEN, ALICE: "Ovicidal effect on the cat flea, Ctenocephalides felis (Bouché), of treating fur of cats and dogs with methoprene" INTERNATIONAL PEST CONTROL, vol. 27, no. 1, 1985, pages 10-13,16, XP002047260 see the whole document --- -/--	1-6

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	CHAMBERLAIN, WILLIAM F. ET AL: "Absorption, excretion, and metabolism of methoprene by a guinea pig, a steer, and a cow" JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY, vol. 23, no. 4, 1975, WASHINGTON,US, pages 736-42, XP002047305 see the whole document	1-6
A	HAWKINS, DAVID R. ET AL: "Fate of methoprene (isopropyl (2E,4E)-11-methoxy-3,7,11-trimethyl-2,4-do decadienoate) in rats" JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY, vol. 25, no. 2, 1977, WASHINGTON, US, pages 398-403, XP002047262 see page 398, the abstract and page 401, figure 3 and table II	1-6
A	PALMA, KATHLEEN G. ET AL.: "Mode of Action of Pyriproxyfen and Methoprene on Eggs of Ctenocephalides felis (Siphonaptera: Pulicidae)" JOURNAL OF MEDICAL ENTOMOLOGY, vol. 30, no. 2, 1993, pages 421-6, XP002048164 see page 421, the abstract see page 426, paragraph 2	1-6

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Information on patent family members

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